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No evidence for trace metal limitation on anaerobic carbon mineralization in three peatland soils

Jason K. Keller*, Jillian Wade

Chapman University, Schmid College of Science and Technology, Orange, CA 92866, USA

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ABSTRACT

Peatlands store roughly one-third of the terrestrial soil carbon and release the potent greenhouse gas methane (CH_4) to the atmosphere, making these wetlands among the most important ecosystems in the global carbon cycle. Despite their importance, the controls of anaerobic decomposition of organic matter to carbon dioxide (CO_2) and CH_4 within peatlands are not well understood. It is known, however, that the enzymes responsible for CH_4 production require cobalt, iron and nickel, and there is a growing appreciation for the potential role of trace metal limitation in anaerobic decomposition. To explore the possibility of trace metal limitation in peatlands, we washed 3 peat soils with either $PbCl_2$, to remove available trace metals, or distilled water. Following these washes, we added trace metals (as $CoCl_2$, $CuCl_2$, $FeCl_2$, $PbCl_2$ and $NiCl_2$) to each soil. We measured anaerobic CH_4 and CO_2 production in laboratory incubations over 4 weeks before adding glucose as a labile carbon source and measuring CH_4 and CO_2 production for an additional 4 weeks. In all 3 soils, neither CH_4 nor CO_2 production were limited by individual trace metals, even following the wash with $PbCl_2$ to remove available metals. Further, in response to the addition of a labile carbon substrate, all soils supported increased rates of CH_4 and CO_2 production without progressive trace metal limitation. Taken together, our findings suggest that individual trace metals may not be limiting to anaerobic decomposition in many peatland soils.

1. Introduction

Peatlands are a diverse group of wetlands that store nearly 500 Pg of carbon in their soils, an estimated one-third of the terrestrial soil carbon (Bridgham et al., 2006; Kolka et al., 2016). Further, peatlands contribute a significant fraction of the flux of methane (CH_4) attributed to global wetland ecosystems (Bridgham et al., 2013; Keller and Medvedeff, 2016). Given that CH_4 has 45-times the sustained-flux global warming potential of CO_2 (on a per mass basis over the 100 year time period; Neubauer and Megonigal, 2015), CH_4 cycling within peatlands can have important implications for the climate. Understanding the role of peatlands in the global climate hinges on our mechanistic understanding of CH_4 and CO_2 dynamics within peatland ecosystems.

At the landscape scale, peatlands are generally classified along a hydrogeomorphic gradient, ranging from precipitation-fed (ombrotrophic) bogs to predominately groundwater-fed (minerotrophic) rich fens. While this gradient is defined by the degree of groundwater influence, a number of other factors, including: pH, dominant vegetation, nutrient availability, cation exchange capacity and trace metal availability also co-vary along this gradient (Bridgham et al., 1996; Kolka et al., 2016). In addition, microbial carbon cycling varies along this gradient with more minerotrophic sites generally exhibiting higher rates of overall carbon mineralization and soils from minerotrophic rich fens producing more CH_4 than soils from ombrotrophic bogs (e.g., Keller and Bridgham, 2007; Updegraff et al., 1995; Ye et al., 2012). Understanding the mechanistic reasons for these differences in CH_4 production among peatland types is crucial for understanding the potential feedbacks between peatland carbon cycling and global climate change.

There is a growing appreciation for the role that trace metals may play in regulating the production of CH_4 in natural ecosystems. In particular, it is known that the enzymes responsible for the production of CH_4 require large amounts of iron, nickel and cobalt (Glass and Orphan, 2012; Jarrell and Kalmokoff, 1988). These trace metals are often found in low concentrations in peatlands (Basiliko and Yavitt, 2001; Gogo et al., 2010; Gorham and Janssens, 2005), which may limit the potential for CH_4 production in these ecosystems. This trace metal limitation may be particularly pronounced in ombrotrophic bogs (often characterized by low CH_4 production) because of small inputs of trace metals from precipitation. Further, *Sphagnum* mosses, which dominate ombrotrophic peatlands, have a high cation exchange capacity and may

* Corresponding author.
E-mail address: jkeller@chapman.edu (J.K. Keller).

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effectively bind trace metals (Gogo and Pearce, 2009a; Thomas and Pearce, 2004). In support of this enhanced trace metal limitation in ombrotrophic peatland soils, Basiliko and Yavitt (2001) reported that a mix of trace metals (iron, nickel, cobalt and sodium) increased rates of CH_4 production in an ombrotrophic bog peatland soil, but not in a minerotrophic fen soil. Similarly, the release of trace metals (e.g., iron, nickel and cobalt) from cation exchange sites (by saturation with lead (Pb^{2+}) or aluminum (Al^{3+})) stimulated CH_4 production in a *Sphagnum*-dominated bog soil, but not in a more minerotrophic fen soil (Gogo and Pearce, 2009b).

These past projects have utilized various 'cocktails' of trace metals (in different combinations and concentrations) to explore for potential limitation of anaerobic carbon cycling. This approach is justified given that carbon mineralization could be co-limited by multiple trace metals; however, as suggested by Basiliko and Yavitt (2001), there is also a need to explore if individual trace metals can limit CH_4 production in peatland soils. In the current project, we tested for limitation of anaerobic carbon mineralization, as CH_4 and CO_2 production, by adding cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), and lead (Pb) individually to 3 peatland soils, ranging from an ombrotrophic bog to a minerotrophic rich fen. We tested for trace metal limitation under multiple experimental conditions in each soil. First, we attempted to induce trace metal limitation by saturating cation exchange sites with $PbCl_2$ and thoroughly washing soils to remove released trace metals. Second, we stimulated rates of anaerobic carbon mineralization by adding a labile carbon substrate (glucose) to test the possibility that progressive trace metal limitation would occur due to increased microbial activity. We hypothesized that (i) the addition of trace metals would stimulate decomposition in ombrotrophic soils more than minerotrophic soils and (ii) trace metal limitation would be exacerbated by both the removal of trace metals following the saturation of cation exchange sites as well as by the increased carbon mineralization following the addition of a labile carbon substrate.

2. Materials and methods

2.1. Site description and sampling

Soil samples for this project were collected from 3 peatlands located on the property of the University of Notre Dame Environmental Research Center in the Upper Peninsula of Michigan, USA. These sites represent a subset of peatlands selected as part of a larger project to represent the ombrotrophic-minerotrophic peatland gradient in this region based on differences in dominate vegetation, soil pH and anaerobic carbon cycling. These sites have been described previously (Ye et al., 2012), and a brief description is provided below. For consistency, we use the same site names utilized by Ye et al. (2012).

"Bog 2" (N46°13′57″, W89°34′7″) is dominated by > 90% cover by *Sphagnum* spp. mosses with scattered short-statured black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggen) and ericaceous shrubs, including: leatherleaf (*Chamaedaphne calyculata* (L.) Moench), small cranberry (*Vaccinium oxycoccos* L.) and bog Labrador tea (*Rhododendron groenlandicum* Oeder). Average water-table depth (reported below hollow surfaces) during the growing season (~May–October) was −16 cm and the pH was 4.1. Soil from Bog 2 had a von Post index of H3 and a rubbed fiber volume content of 38 ± 10% (values from Ye et al., 2012; mean ± 1 standard error) suggesting that the peat at this site was less decomposed than at the other sites. "Acidic Fen" (N46°12′48″, W89°30′2″) has a *Sphagnum* spp. lawn with minimal cover from other species in the area of sampling. The average water-table depth was −10 cm and the pH was 4.1. Soil from Acidic Fen had a von Post index of H3/H4 and a rubbed fiber volume content of 35 ± 9% (values from Ye et al., 2012). "Rich Fen" (N46°13′27″, W89°29′53″) is dominated by the upright sedge (*Carex stricta* Lam.) although leatherleaf shrubs are also present on tussocks. This site was consistently flooded with ~30 cm of standing water during the 2009 growing season. The average pH was 5.9 and soil from Rich Fen had a von Post index of H5 and a rubbed fiber volume content of 15 ± 5% (values from Ye et al., 2012), suggesting that this peat was the most decomposed of the sites studied.

Soil were collected from 30 cm below the water table measured in the field in each peatland in August of 2009 using 10-cm diameter PVC cores. Cores were 30 cm in length and were inserted into the soil with the aid of a serrated knife to minimize compaction. Cores were extruded into large Ziploc bags, frozen and shipped to Chapman University in Orange, CA. Prior to the initiation of this experiment, individual cores were allowed to thaw and large roots and living vegetation were removed by hand in the ambient atmosphere. The remaining root-free peat was refrozen. The length of time a core was thawed varied, but was generally < 1 week.

2.2. Determination of water-extractable cations and cation exchange capacity

Concentrations of water-extractable cations were measured by adding 20 g of field-moist, root-free peat to 20 mL of deionized water and shaking at 200 rpm for 1 h. Following the extraction, the slurry was centrifuged at 4100 rpm for 5 min and the supernatant was filtered through a P8 qualitative filter and frozen until analysis for cations at the Soil and Plant Tissue Testing Laboratory at the University of Massachusetts.

Soil cation exchange capacity (CEC_{Ca}) was determined by compulsive exchange with Ca^{2+} (Gogo and Pearce, 2009a). Briefly, 0.15 g of air-dried peat was washed twice with 20 mL of 0.01 M HCl for 5 min to remove background levels of Ca^{2+}. Following each wash, the samples were centrifuged at 4100 rpm for 5 min and the supernatant was discarded. After both HCl washes, the remaining soil was washed twice with deionized water. Subsequently, the soil was saturated with 20 mL of 0.01 M $CaCl_2$. This slurry was centrifuged for 5 min at 4100 rpm and the supernatant was discarded. After the $CaCl_2$ saturation, the remaining soil was washed twice with deionized water. Finally, 20 mL of 0.01 M HCl was added to the soil three times. After each addition, the soil was centrifuged at 4100 rpm for 5 min and the supernatant was collected. After all three washes, the combined supernatant was brought to 100 mL with 0.01 M HCl and this solution was analyzed for Ca^{2+} at the Soil and Plant Tissue Testing Laboratory at the University of Massachusetts.

2.3. Experimental design

For logistical reasons, peat from each site was treated separately in this experiment (i.e., the treatments described below were applied to each peat at a separate time). This approach was appropriate as our intention was to focus on the importance of trace metals on carbon mineralization within a peatland while focusing on the more qualitative patterns (i.e., stimulation or inhibition by a given trace metal) between sites.

2.3.1. Wash treatments

To explore the role of trace metals bound to cation exchange sites, soils were initially washed with either deionized water or $PbCl_2$. The water wash treatment was intended to remove dissolved cations. In contrast, the $PbCl_2$ wash treatment was intended to release soil-bound cations by saturating cation exchange sites with Pb^{2+} (Gogo and Pearce, 2009b), and thus to induce trace metal limitation. Both wash treatments also removed dissolved organic matter, with important implications for the interpretation of our results (see Discussion section for additional details). For each replicate soil core, 100 g of field-moist peat was added to a Mason jar, amended with 100 mL of 2 mM $PbCl_2$ or 100 mL of deionized water, and shaken at 200 rpm for 1 h. The peat was then transferred into 50-mL centrifuge tubes and centrifuged at 3000 rpm for 5 min. The resulting supernatant was discarded and the

peat was returned to the Mason jars and washed an additional 5 times with 100 mL of deionized water. Each wash cycle included shaking at 200 rpm for 10 min, transferring to centrifuge tubes, centrifugation at 3000 rpm for 5 min, discarding of the supernatant, and returning the peat to the Mason jar. The supernatant resulting from the fifth wash cycle tested negative for chloride (Chloride Test Kit, Model 8-P, Hach Company, Loveland, CO), suggesting that the chloride from the $PbCl_2$ wash had been removed. Following the final wash, the peat was allowed to sit overnight at 4 °C. The following morning, the peat was centrifuged once more at 3000 rpm for 5 min and the supernatant was discarded.

Ten gram subsamples of peat from each replicate core were added to twelve 160-mL serum bottles (6 contained $PbCl_2$ washed peat, 6 contained deionized water washed peat) along with 10 mL of deionized water which had been bubbled with N_2 for 15 min. The pH of each slurry was measured after 30 min. Subsequently, the bottles were capped with gray butyl septa and the headspaces were flushed with N_2 for 15 min to establish anaerobic conditions. Additional subsamples of washed peat were dried at 60 °C for 48 h to determine percent moisture content.

2.3.2. Phase I: equilibration

All soils were incubated for 2 weeks in the dark at 22 °C. This equilibration phase was intended to allow microbial communities to re-establish after the wash treatments but before trace metal treatments were added to the soils. Headspace CH_4 and CO_2 concentrations were measured using a gas chromatograph (SRI 8610C, SRI Instruments, Torrance, CA) with a flame ionization detector and an in-line methanizer (to convert CO_2 to CH_4) on days 1, 3, 5, 7, 10 and 14. Dissolved CH_4 and CO_2 were calculated using Henry's Law adjusting for solubility, temperature and pH (Drever, 1997). Headspace and dissolved pools of both gases were summed to calculate total production.

2.3.3. Phase II: trace metal amendment

Following the equilibration phase, the bottles were opened in an anaerobic chamber (~95% N_2 and < 5% H_2 headspace; Coy Laboratory Products, Inc., Grasslake, MI) and the pH of each slurry was measured. Each bottle was amended with 10 mL of 0.2 mM trace metal solutions of $CoCl_2$, $CuCl_2$, $FeCl_2$, $NiCl_2$, or $PbCl_2$. The use of only divalent cations for this experiment normalized the amount of chloride added in each treatment. The amount of trace metals added by these treatments was 0.2 μmol g wet peat^{-1} (equivalent to ~1–5 μmol gdw^{-1}, depending on the moisture content of the different peat soils). These concentrations are higher than those added by Basiliko and Yavitt (2001) who added 0.02, 0.04 and 0.05 μmol g wet peat^{-1} of Co, Fe and Ni, respectively. They are more comparable to the concentrations added by Williams and Crawford (1984) who added 0.5 and 3.7 μmol g wet peat^{-1} of Co and Fe, respectively. All trace metal solutions were degassed with N_2 for five minutes prior to addition. Ten milliliters of degassed, deionized water were added to the control treatment. The pH was recorded 30 min after treatment amendment. Bottles were capped, removed from the anaerobic chamber and flushed with N_2 for 15 min to ensure anaerobic conditions. The amended peat slurries were allowed to incubate in the dark at 22 °C for 4 weeks. Methane and CO_2 production were measured on days 1, 3, 5, 7, 10, 14, 21 and 28 as described above.

2.3.4. Phase III: trace metal and labile carbon amendment

At the end of Phase II, all bottles were opened in an anaerobic chamber and pH was recorded. Each bottle was then amended with a second 10 mL treatment of the appropriate 0.2 mM trace metals solution which also contained 10 mM of glucose. The glucose treatment added 0.6 mmol of carbon to the bottles, which was approximately 20-, 5- and 3-times the amount of carbon released as both CH_4 and CO_2 in Phase I and Phase II of the experiments in the Bog 2, Acidic Fen and Rich Fen soils initially washed with distilled water, respectively (data from Fig. 1). These treatments were designed to explore the possibility that trace metal limitation was only present when labile carbon was not limiting to anaerobic carbon mineralization. The pH of each bottle was recorded after 30 min, and the bottles were recapped and flushed with N_2 for 15 min before being returned to a dark, 22 °C incubator. Methane and CO_2 production were measured on days 1, 3, 5, 7, 10, 14, 21, and 28 as described above. After the final phase of the incubation, the bottles were opened and the final pH was recorded.

2.4. Data analysis

We present cumulative CH_4 and CO_2 production (μmol C gdw^{-1}) in the unamended control treatment at the end of each experimental phase to provide a context for rates of carbon mineralization in our experiment. The effects of the different initial wash treatments (i.e., the deionized water wash and the $PbCl_2$ wash) on cumulative CH_4 and CO_2 production at the end of each experimental phase were explored using independent samples *t*-tests within each soil. Differences in CEC_{Ca} and water-extractable cation availability between soils were analyzed by one-way ANOVA followed by Fisher's LSD. Linear regressions were used to explore relationships between pH and CH_4 and CO_2 production during an experimental phase.

To examine the effect of trace metal additions on rates of CH_4 and CO_2 production, we calculated the CH_4 Response and the CO_2 Response for each soil during Phase II (trace metal amendment phase) and Phase III (trace metal and labile carbon amendment phase) as follows:

$$\text{Response} = [\,(\text{Treatment Rate}+1) - (\text{Control Rate}+1) /(\text{Control Rate}+1)\,] * 100\ .$$

where Treatment Rate was the cumulative production of CH_4 or CO_2 in a soil amended with a trace metal and Control Rate was the cumulative production of the same gas in the unamended control soil from the same replicate core. In this approach, positive response values indicate a stimulation of CH_4 or CO_2 production in response to trace metal amendment and negative values indicate an inhibition. To test for significant effects of trace metal additions in Phase II (trace metal amendments) and Phase III (trace metal and labile carbon amendment), we used a one-sample *t*-test to compare the observed response to 0. Given the large number of comparisons within each peat type (40 individual tests), we utilized a Bonferroni correction to set $\alpha = 0.00125$. This approach is admittedly conservative, but given the lack of stimulation observed (see below), we felt it was appropriate.

3. Results

Cation exchange capacity (CEC_{Ca}), measured as Ca^{2+} exchange, was higher in the minerotrophic Rich Fen soil (0.284 ± 0.023 mmol gdw^{-1}) than in the more ombrotrophic Bog 2 (0.190 ± 0.004 mmol gdw^{-1}) and Acidic Fen (0.154 ± 0.004 mmol gdw^{-1}) soils (Table 1). Total water-extractable cations ranged between 4.92 ± 0.61 μmol gdw^{-1} in the Bog 2 soil and 6.30 ± 0.38 μmol gdw^{-1} in the Acidic Fen soil, but did not differ between the three soils (Table 1). Differences in individual water-extractable cations between soils varied for different cations. Calcium and Mg were highest in the Rich Fen soil; Al was highest in the Acidic Fen soil; K was lowest in the Rich Fen soil and Fe was lowest in the Bog 2 soil. Water-extractable P was similar in all soils (Table 1).

Cumulative CH_4 production was generally highest in the Rich Fen soil and comparable in the Bog 2 and Acidic Fen soils across all experimental phases (Fig. 1A, C and E). Cumulative CO_2 production was also highest in the Rich Fen soil during the equilibration phase and the trace metal amendment phase, although the magnitude of the differences between soils was less pronounced for CO_2 than it was for CH_4 (Fig. 1B, D). In the presence of labile carbon, cumulative CO_2 production was highest in the Bog 2 soil and comparable between the Acidic Fen and Rich Fen soils (Fig. 1F).

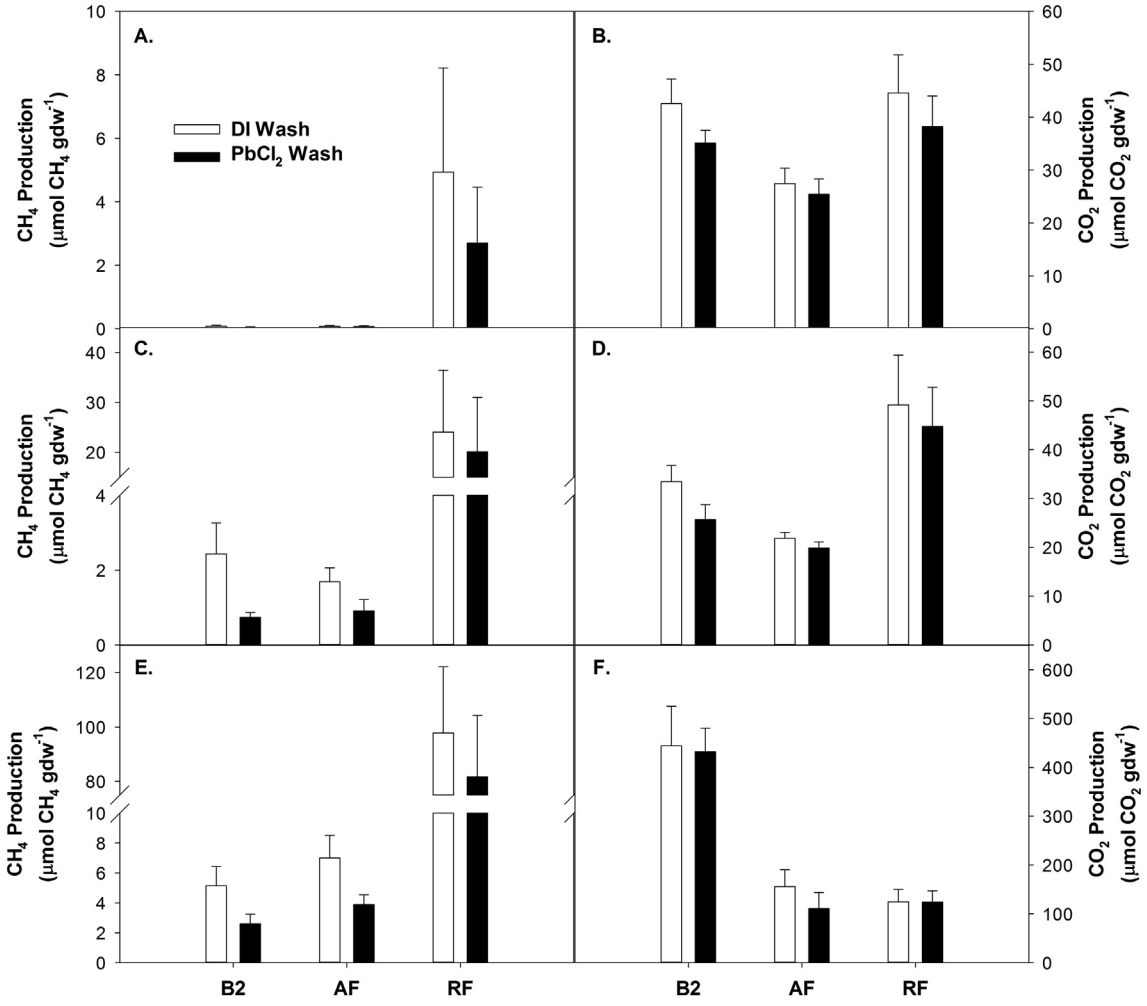

Fig. 1. Mean (± 1 SE; $n = 5$) cumulative CH_4 and CO_2 production in Bog 2 ("B2"), Acidic Fen ("AF") and Rich Fen ("RF") soils which were initially washed with deionized (DI) water or $PbCl_2$; but were not amended with additional trace metals (i.e., the control treatment). Cumulative gas production over the 2-week equilibration phase (A. and B.); during the 4-week trace metal amendment phase (C. and D.); and over the 4-week trace metal and labile carbon amendment phase (E. and F.) are shown. Note that the axes scales are different for each panel.

Table 1
Mean (± 1 SE) CEC_{Ca} and water-extractable cations in three peatland soils. Concentrations of Zn, B, Mn, Cu and Pb were also measured but were below 0.01 μmol gdw^{-1}. Total is the sum of all measured extracted cations. Different letters reflect differences between the soils ($p < 0.05$) based on one-way ANOVA followed by Fisher's LSD.

	CEC_{Ca}	P	K	Ca	Mg	Fe	Al	Total
	mmol gdw^{-1}	μmol gdw^{-1}						
Bog 2	0.190 ± 0.004^{A}	0.76 ± 0.24	2.16 ± 0.46^{B}	1.04 ± 0.08^{A}	0.44 ± 0.03^{A}	0.10 ± 0.01^{A}	0.40 ± 0.10^{A}	4.92 ± 0.61
Acidic Fen	0.154 ± 0.004^{A}	0.95 ± 0.20	2.43 ± 0.40^{B}	1.14 ± 0.05^{A}	0.49 ± 0.02^{A}	0.20 ± 0.04^{B}	1.06 ± 0.13^{B}	6.30 ± 0.38
Rich Fen	0.284 ± 0.023^{B}	0.41 ± 0.14	0.93 ± 0.21^{A}	2.84 ± 0.16^{B}	0.82 ± 0.07^{B}	0.23 ± 0.02^{B}	0.67 ± 0.12^{A}	6.00 ± 0.57

Over the course of the experiment, rates of CH_4 production increased in the unamended soils (Fig. 1A, C), and showed an increase in response to the labile carbon amendment (Fig. 1E). However, the stimulation of CH_4 production by the labile carbon addition was more dramatic in the Rich Fen soil than in either the Bog 2 or Acidic Fen soils (Fig. 1E). In contrast, rates of CO_2 production decreased over the course of the experiment with comparable cumulative CO_2 production during the 2-week equilibration phase (Fig. 1B) and the 4-week trace metal amendment phase (Fig. 1D). The addition of labile carbon stimulated CO_2 production in all soils, but this effect was most pronounced in the Bog 2 soil (Fig. 1F).

In all soils, cumulative CH_4 and CO_2 production were lower in soils that were washed with $PbCl_2$ than in soils that were washed with deionized water during the experimental phase (Fig. 1). These differences were most pronounced for CH_4 production in the Bog 2 and Acidic Fen Soils, where cumulative CH_4 production in the $PbCl_2$ wash treatment was between 30% and 55% of the deionized water washed treatment during the trace metal amendment phase and the trace metal and labile carbon amendment phase (Fig. 1C and E). While the reduction in CH_4 and CO_2 production in response to the $PbCl_2$ wash was consistent across all soils, the differences between the deionized water wash and the $PbCl_2$ wash were not significant in any experimental phase for any soil ($p \geq 0.08$ for all independent t-tests).

There was little evidence of trace metal limitation in these peatland soils (Figs. 2–4). In the Bog 2 soil, the addition of trace metals generally inhibited CH_4 production (Fig. 2). This inhibition was more pronounced in the deionized water washed soils than the $PbCl_2$ washed soils when trace metals were added alone (Fig. 2A), but the difference between

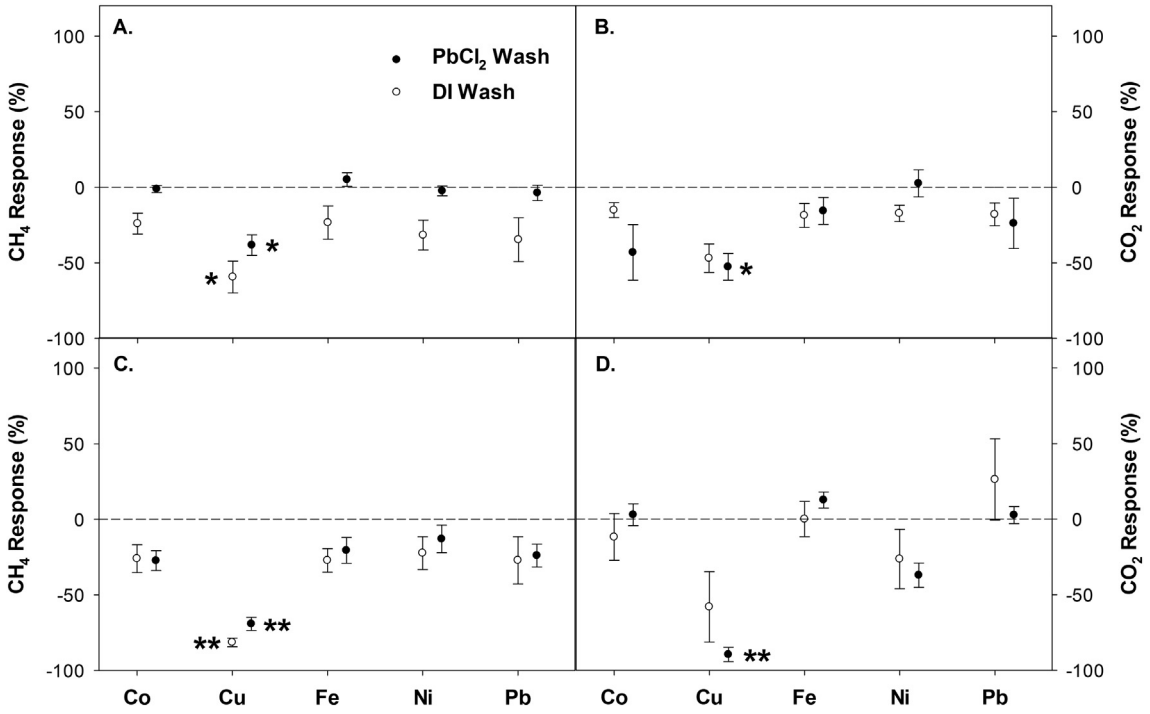

Fig. 2. Mean (± 1 SE; $n = 5$) response of cumulative CH_4 and CO_2 production in the Bog 2 soil, which was initially washed with deionized (DI) water or $PbCl_2$. The responses during the 4-week trace metal amendment phase (A. and B.) and over the 4-week trace metal and labile carbon amendment phase (C. and D.) reflect stimulation (positive values) or inhibition (negative values) by divalent cations, relative to unamended controls. Asterisks indicate a significant effect of the trace metal amendment based on a one-sample *t*-test (* = $p \leq 0.005$; ** = $p \leq 0.001$).

wash treatments disappeared when trace metals were added with a labile carbon source (Fig. 2C). Methane production was lowest following the amendment of Cu in the Bog 2 soil, with a CH_4 response of − 38 and − 59% for the deionized water and $PbCl_2$ washed soils, respectively, in the trace metal amendment (Fig. 2A) and − 69 and − 81% for the deionized water and $PbCl_2$ washed soil when trace metals were added with a labile carbon source (Fig. 2C). There was also no significant stimulation of CO_2 production in the Bog 2 soil in response to the addition of trace metals. Similar to CH_4 production, the most dramatic inhibition of CO_2 production was in response to the addition

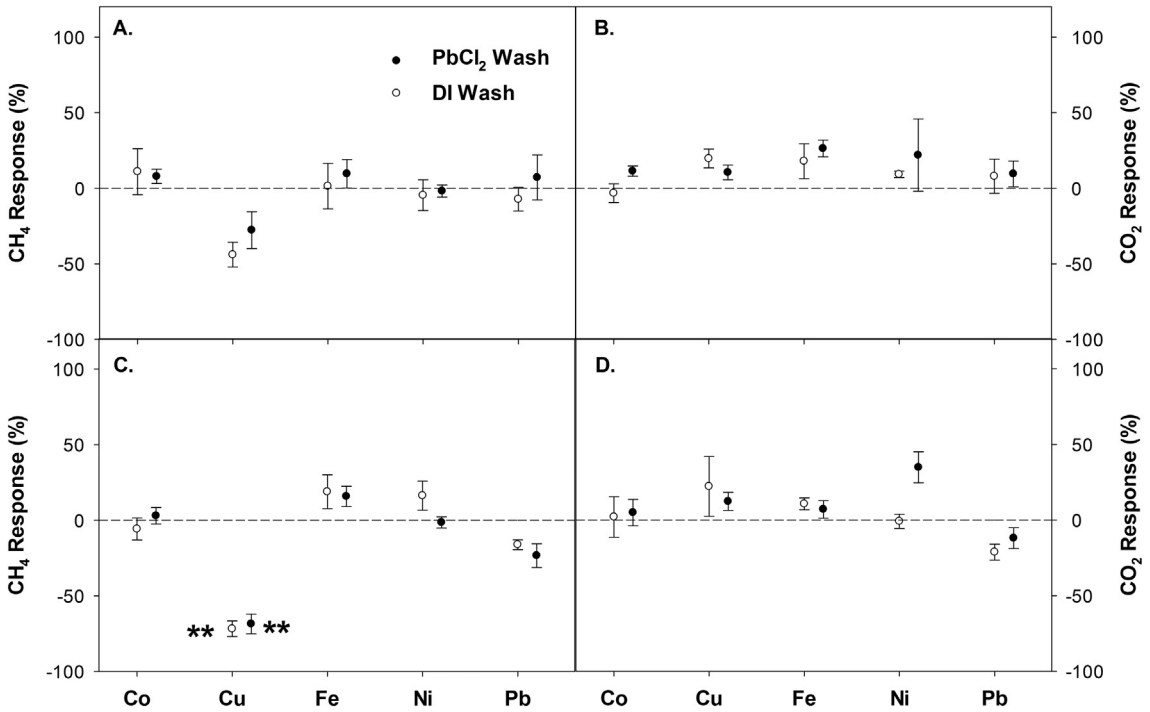

Fig. 3. Mean (± 1 SE; $n = 5$) response of cumulative CH_4 and CO_2 production in the Acidic Fen soil, which was initially washed with deionized (DI) water or $PbCl_2$. The responses during the 4-week trace metal amendment phase (A. and B.) and over the 4-week trace metal and labile carbon amendment phase (C. and D.) reflect stimulation (positive values) or inhibition (negative values) by divalent cations, relative to unamended controls. Asterisks indicate a significant effect of the trace metal amendment based on a one-sample *t*-test (* = $p \leq 0.005$; ** = $p \leq 0.001$).

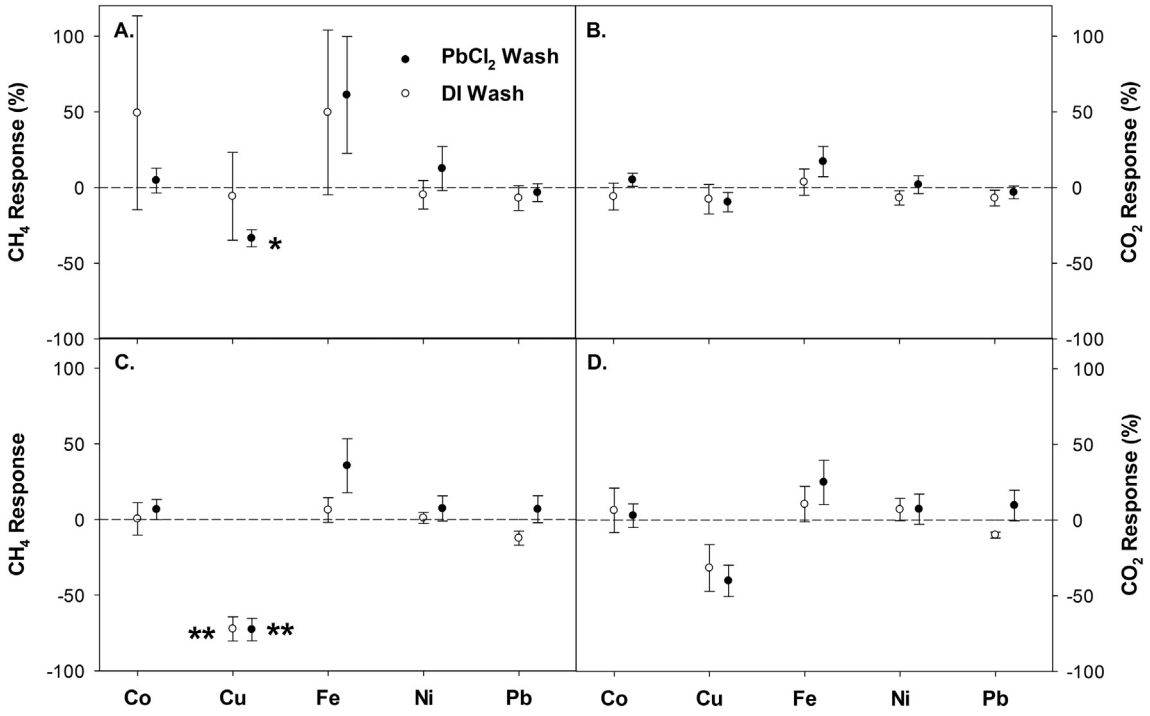

Fig. 4. Mean (± 1 SE; $n = 5$) response of cumulative CH_4 and CO_2 production in the Rich Fen soil, which was initially washed with deionized (DI) water or $PbCl_2$. The responses during the 4-week trace metal amendment phase (A. and B.) and over the 4-week trace metal and labile carbon amendment phase (C. and D.) reflect stimulation (positive values) or inhibition (negative values) by divalent cations, relative to unamended controls. Asterisks indicate a significant effect of the trace metal amendment based on a one-sample *t*-test (* = $p \leq 0.005$; ** = $p \leq 0.001$).

of Cu in the Bog 2 soil (Fig. 2B, D); although the inhibitory effect of Co was almost as dramatic for the $PbCl_2$ washed soil in the absence of labile carbon (Fig. 2B).

In the Acidic Fen soil, there were few increases in CH_4 or CO_2 production in response to trace metal amendments (Fig. 3). In general, CH_4 production showed few responses to trace metal amendments with the exception of an inhibition by Cu in both the absence (− 44% and − 28% for deionized water washed and $PbCl_2$ washed soils) and presence of labile carbon (− 72% and − 69% for deionized water washed and $PbCl_2$ washed soils; Fig. 3A, C). Average CO_2 production was frequently stimulated by trace metal addition; however, this stimulation was relatively minor. The most pronounced increases were observed in response to the addition of Fe in the absence of labile carbon and in response to the addition of Ni in the presence of labile carbon (Fig. 3B and D, respectively).

The Rich Fen soil exhibited the highest average stimulation of CH_4 production in response to the addition of Co (deionized water wash only) and Fe (both washes) in the absence of labile carbon (Fig. 4A). However, these results were driven by a single replicate core with low rates of CH_4 production in the control treatment. Similar to the other soils, there was an inhibitory effect of Cu in the Rich Fen soil, although this effect was not seen in the deionized water washed soil in the absence of labile carbon (Fig. 4A and C). There were few effects of trace metal amendment on CO_2 production in the Rich Fen soil, although there was an inhibitory effect of Cu in the presence of labile carbon (Fig. 4B and D).

4. Discussion

Contrary to our initial hypotheses, neither CH_4 nor CO_2 production were limited by any of the individual trace metals investigated in these peatland soils. The lack of stimulation of anaerobic decomposition by trace metals was consistent across three peatland soils representing a range of trace metal availabilities and cation exchange capacities (Table 1) as well as a range of cumulative carbon mineralization (Fig. 1). Even following a wash with $PbCl_2$ to remove available metals, the addition of individual trace metals did not lead to an increase in decomposition. Similarly, these soils supported increased rates of CH_4 and CO_2 production in response to the addition of a labile carbon substrate (glucose) without progressive trace metal limitation (Figs. 2–4).

These results appear contrary to past studies which have suggested a potential for trace metal limitation in peatlands, particularly ombrotrophic soils (Basiliko and Yavitt, 2001; Gogo and Pearce, 2009a; Gogo and Pearce, 2009b). It is worth noting, however, that trace metal limitation is not universal in these past peatland studies. For example, a trace metal solution (added simultaneously with a nitrogen/phosphorus solution and containing magnesium, iron, manganese, zinc and cobalt) inhibited CH_4 production in soil from a *Sphagnum*-dominated transition fen (Williams and Crawford, 1984). Even the trace metal solution (containing nickel, cobalt and iron) that resulted in 2- to 3-fold increases in CH_4 production in two bog soils had no stimulatory effect, or even an inhibitory effect, in 3 other peatland soils (Basiliko and Yavitt, 2001). Trace metals when added alone, as in this study, or in combination do not always result in the stimulation of CH_4 production in peatlands and the mechanisms for the variable responses in different peats remain elusive.

Gogo and Pearce (2009a) attributed the stimulation in CH_4 production in response to a wash with $PbCl_2$ to the release of trace metals from cation exchange sites; however, they also highlighted the fact that this apparent trace metal stimulation disappeared in the presence of labile carbon. Similar to our results, this suggests that carbon may be the fundamental limiting element for anaerobic decomposition. Given the apparent role of carbon limitation, it is important to note that the initial wash treatments used in this experiment also removed dissolved organic matter from the peat soils. There is mounting evidence that dissolved organic matter can be the primary source of carbon for methanogens in peatland soils. For example, the radiocarbon age of CH_4 is frequently more similar to dissolved carbon than solid-phase carbon in peatland ecosystems (e.g., Chanton et al., 2008; Tfaily et al., 2014;

Wilson et al., 2016). Even after adding a labile carbon source in the form of glucose in Phase III of the current experiment, it appears that the active microbial community was able to mineralize that carbon using the available trace metals, even following the $PbCl_2$ wash treatment (Figs. 2–4).

Another potential artifact of our experimental design was that the amendment of trace metals generally decreased the pH of the incubations (Supplemental Table 1), possibly due to the release of H^+ from cation exchange sites in the presence of trace metals. Similar decreases in pH have been observed in past trace metal experiments (Gogo and Pearce, 2009a; Gogo and Pearce, 2009b). This acidification was more pronounced in the more ombrotrophic Bog 2 and Acidic Fen soils. Linear regressions suggest that in Bog 2 soil, these differences in pH explained between 34% and 40% of CH_4 and CO_2 production in the trace metal amendment and trace metal and labile carbon amendment phases. In the Acidic Fen soil, differences in pH explained 40% of CH_4 production and 29% of CO_2 production during the trace metal amendment and labile carbon amendment phase. The small differences in pH in response to trace metal amendments did not explain difference in CH_4 and CO_2 production in the Rich Fen soil (Supplemental Table 2). The important role of pH in the more ombrotrophic soils is consistent with the recognized role of soil pH in regulating peatland carbon cycling and CH_4 production in particular (e.g., Dunfield et al., 1993; Ye et al., 2012). Future studies should consider the potential for these pH effects to mask trace metal limitation as the inhibitory effects of lower soil pH would counteract potential stimulation by trace metals.

The most consistent effect of trace metal additions was the reduction in both CH_4 and CO_2 production in all soils by Cu (Figs. 2–4). This reduction was dramatic - up to 81% reduction in CH_4 production - and suggests a direct inhibitory effect of Cu. The toxic effects of Cu on microbial activity are well documented (Beveridge et al., 1997), and a negative correlation between CH_4 emissions and dissolved Cu in rice soils suggest that the inhibitory effects of Cu may extend to the production of CH_4 in wetland environments (Jiao et al., 2005). While determination of the Cu concentrations necessary to inhibit microbial processes was beyond the scope of this study, it is important to note that the amount of Cu added to peats in the trace metal amendment and the trace metal and labile carbon amendment phases of this experiment far exceed the water soluble Cu in these soils (Table 1). However, Thomas and Pearce (2004) demonstrated that $CuCl_2$ treatment at ~10-times the concentration used in this study stimulated CH_4 production in bog from a peat soil, but only at intermediate depths. They suggested that this was due to the displacement of other trace metals coincident with copper binding to CEC sites in this soil.

Taken together, our results suggest that individual trace metals do not limit anaerobic decomposition in many peatland soils. The lack of progressive limitation in response to the removal of metals by the $PbCl_2$ wash and the addition of glucose suggest increased rates of decomposition are not likely to be limited by trace metals in these systems.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2017.11.001.

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